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TRANSLATION NO. 77

DATE: Sept 1968

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REC 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

2/11
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INVESTIGATIONS OF NATURAL AND ARTIFICIAL IMMUNITY AGAINST ANTHRAX

(From the Hygienic Institute of the German University at Prague)

(Chief: Professor Hueppe)

XI. First Report About Anthrax Immunization Inoculations on Sheep.

By: Professor Dr. Oskar Bail, Assistant of the Institute.

The following is the first verbatim transcript of a report of the Austrian Ministry of the Interior (with the exception of a few minor changes), which approved the means of the investigations and to which office we are gratefully indebted. Although ^aconclusive opinion has not been reached yet, i.e. the duration of the immunity following inoculation, new points of view have arisen which justify publication. As the housing of the sheep was impossible in outside locations, the institute had to provide somewhat inadequate accommodations, for the test animals. Although this had the advantage of providing a constant, exact supervision, it also presented considerable inconveniences. All sheep were pasture animals and arrived in an exhausted condition. Throughout the hottest part of the summer they were placed in narrow stalls, lacking adequate lighting and ventilation. The pretreatment had to be started at a time before the animals had recuperated from the strenuous trip. There was no possibility to exercise the sheep and their feed was also inequitable.

Regarding the basis of the immunization methods, Chapter X dealing with these investigations contained more detailed information. The question is an immunization against anthrax by means of "Lysin"

(as per Kruse), which can be obtained from the sterilized edema liquid from anthrax infected animals. Through this, after the developed conception of "antilysin" is formed in the body of the treated animals and this is immunized with live bacillus before the infection.

Tests on rabbits and pre-tests on sheep have proved the correctness of this assumption. The animals treated in the above mentioned manner did not only become immune themselves, but also produced serum which could protect normal animals. Theoretically, this serum possessed none of the qualities of a bactericidal immune serum although it had a decisive immunizing effect.

In conjunction with the reported tests on sheep, further details are given below; these details have been partially acquired during the investigations. The entire explanation depicts one outstanding fact, i.e., a special immunization (active immunity) is achieved through the edema treatment. This differentiates itself from the other methods used, viz. the Pasteur with its deviations and the Sobernheim(?) which represent a combination of active and passive immunization, mainly because the participation (activity) of live bacillus (virulent as well as attenuated) is completely eliminated.

CHART I

<u>Mr.</u>	<u>Pre-Treatment</u>	<u>Infection</u>	<u>Result</u>
IV	8 June 5 ccm rabbit edema sub-cutaneously into right thigh	right thigh	Pre-treatment endured without any reaction, but on 12 June the animal developed a paralysis in the hind legs and symptoms of stagers. 33 hours after the inoculation, slight reddening of the place of infection, but no outspoken edema. Dies 43 hours after the infection. No edema, but some bloody pus on the place of injection. Spleen very slightly enlarged. Abundant bacillus in blood and organs. In the brain a worm shaped blister (bubble) filled with almost clear liquid.
V	8 June, 5 ccm rabbit edema sub-cut. right thigh 12 June repeat.	right thigh	Pre-treatment endured without any reaction. 48 hours after injection severely ill, with- out localized symptoms. 19 June remarkable improvement 23 June recovered.
VI	18 June, 10 ccm rabbit edema sub-cut. on right thigh, 12 June repeat	right thigh	Pre-treatment as well as infection endured without any reaction.
VII	Same as VI	left thigh	Same as above
VIII	8 June, 15 ccm Rabbit edema on right thigh	right thigh	Same as above
XIII (controlled animal)	-	right thigh	24 hrs after the infection evident, 36 hrs very pro- nounced bloody edema. Death within a period of 42 hrs. cause - anthrax.

10 June 2/80 Bac. Sub.

The first test covers 6 sheep of an ordinary Hungarian breed. Five of these animals, as illustrated in Chart I, were pre-treated with edema of anthrax infected rabbits. The last animal (Nr. XIII) served as a control animal for this test as well as for a simultaneously conducted test, (transmitted (passive) immunization) as expressed in Chart II. This test was made with 4 sheep of the same breed. The serum used for this purpose stems from a sheep III(3) which has been treated with edema for a long time. (s.a.a.b. p.271)

CHART II
(Transmitted Immunity with Serum from Sheep III)

Nr.	Serum Treatment	Infection	Results
IX	16 June 5 ccm serum subcutaneously right thigh	1 hr. later 2040 Bac. on left thigh	On 19 June shows a small pea sized hard infiltrate on the left thigh which diminished by 27 June. The general condition did not show any disturbance.
X	16 June, 10 ccm like IX	Like IX	19 June slight edema, which is well definable and hardened on the following day; accompanied by considerable swelling of glands, everything disappeared and on 27 June the animal was back to normal. The general condition did not show any disturbance.
XI	16 June, 5 ccm to which 2040 bac.were added immediately prior to inoculation subcutaneous right thigh		Without any reaction.
XII	16 June, 10 ccm Serum- rest like XI		Without any reaction
XIII (controlled animal)	-		See Test Chart I

The 3rd Test follows. This test was made to ascertain whether or not the achieved immunity of the sheep was relevant. As all animals could not be inspected because of lack of sufficient space, only spot checks were made. Unfortunately, due to a dosage error the amount of bac. was somewhat small.

CHART III

Nr.	Infection	Results
V	30 June, 592	Without any reaction
VI	Bac. sub-	
X	cutaneous	
XI	left thigh	
XIV	same as above	Dies on the 14th day with excessive edema and typical anthrax.

In regard to the transmitted immunity, Serum from Sheep III, as observed on rabbits, also completely protected sheep or in some instances reduced the infection to slight local symptoms. The severity (gravity) of the infection and the extremely great (sensitivity) sensitiveness of the used (employed) breed is clearly (observed) confirmed by the short life span of the controlled sheep.

A precise evaluation of the effect of the serum was not included in the testing program. The plan merely tended to prove that the same results already determined on rabbits, also applied to sheep. However, one can hardly doubt that the smallest protective quantity could be lower than 5 ccm. This is supported by the absence of any reaction, at least when bac. serum is simultaneously injected (inoculated.) It would be erroneous to assume that through separate injection edema etc. was observed and that the serum bactericidal would cause the same effects as the "typhus immunization serum". Actually any trace of bacteria distinction was missing.

(employed)
Two (2) ccm of the fresh, 2-hour-old serum were mixed with 0.002 ccm of the cultures deluted in bouillon for the inoculation of the sheep. This was maintained at 37°. Each loop contained: at the beginning 184, after 24 hours 291 and after 5 hours 10,000, after 24 hours 0' bacilli.

Without assuming a fast destruction of bacilli, it is easy enough to offer an explanation.

(Somewhat surprising was the result that the animals protected by serum even when the first infection (inoculation) (like with XI) showed no reaction.)

Somewhat surprising was the result that the animals protected by serum were still unreceptive 14 days later although the first inoculation (infection) (like with Nr. XI) had caused no reaction. Contrary to this, rabbit tests have shown an immunity of not more than a week's duration and even then when an alike serum was used (rabbit immune serum for rabbits). As these conditions (provisions) could be of practical importance, it was planned to extend these tests.

The main objective is the achievement of a homogeneous immunity; based on past experience, only this type of an immunity provides a longer period of protection. However, in this respect, (the actual duration) it is impossible to come to a conclusive decision at the present time. Test I proves (determines) that edema treatments are completely (totally) successful. Test III shows that a total immunity is still maintained 3 weeks later (true enough, however, against a smaller dose of bacilli). The only loss of these sheep treated with rabbit edema

was the animal which received the smallest dose (amount) of edema. Although the sheep had also another disease, we must still attribute the lack of immunity to the insufficient dose of the edema inoculation because another sheep, which was administered the next highest dose survived in spite of suffering from a severe illness. Both sheep VI and VII which had pre-treatment and bac. inoculations on similar and different parts of their bodies prove that it is a question of a general and not a localized insensitiveness, and finally sheep VIII established (determined) that even a single inoculation of a sufficiently large dose of edema could suffice. Naturally, it is not simple to determine the necessary size of the dose and can be done only by counting cubic centimeters.

Further, all sheep prove that edema inoculations can be endured without any damage or even local or general manifestations.

Sheep from Siebenburgen were used for the following two tests. These were, in view of their wool, better grade animals. Their hardiness, however, was considerably higher than the one of the breed previously used. The results (statistics) were different in many respects. The infection (inoculation) was heavier (greater) not only on account of the number of bacilli, but also because the cultures used had gone two or three times through the body of the sheep. Besides, to simulate natural conditions during which the infection is mainly caused through spores, agar cultures were used.

The main difference was in the manner of the pre-treatment. (This time edema liquid of sheep suffering from anthrax was used and not the

liquid from diseased rabbits, in other words a homogeneous substance.)

This time the edema liquid used was not from diseased rabbits but from sheep suffering from anthrax, in other words a homogeneous substance. The experiences of the last years have made it more and more probable that this is a vital point in the immunity research which has not been sufficiently investigated. We wish to refer here briefly to the investigations conducted by Wood and Grassberger and Schattenfroh relating to toxines, as well as to the research made by Ehrlich and his school for better (protective) immunity effects of homogeneous bactericidal immune sera. Apparently only loosely related, such observations point to a vital, still to be investigated (condition) connection, "a secret loss."

Obviously this pertains also to the anthrax immunity. It is extremely difficult to immunize guinea pigs with rabbit edema and then only against slight infections. It is much easier to utilize the edema or peritoneal exudate of guinea pigs suffering from anthrax. Besides, the protective effect of a serum from a rabbit which was treated with guinea pig edema is far higher for guinea pigs than when the test (serum) animal is immunized with rabbit edema. This result is positive although the pertinent investigations are not yet concluded (completed). Taking into consideration the excessive sensitiveness of guinea pigs to anthrax this is easily explainable.

A "Lysin Theory" of the anthrax immunity can put up with the above fact, at least temporarily. It is evident that the "protective matter" (which the bacillus secretes as a "matter exchange substance") (like

the tetanus bacillus) must depend on "protective devices," which have to be combat^{ed} (overcome) within the animal body. Though similar in ^{many} ways, in some instances the anthrax "lysin" of a rabbit varies from the substance derived of a sheep. Hence the latter may become important for the immunization.

This trend of thought, whose theoretical justification cannot be denied, governed the following test, yet, at first in a wrong manner (way). It was assumed that homogeneous edema of sheep could be easily "consumed" (worked up) by sheep and therefore immunity would set in at an early stage. Based on this positive assumption an extensive test was prematurely started and turned out accordingly. Besides using (as inoculation liquid) pure edema, we also employed a mixture of edema and blood of a sheep suffering from anthrax. (See Chart IV)

If we consider this test primarily as an immunity inoculation attempt, there is little to report: of 6 pre-treated animals, 5 died of anthrax, whereas the sixth survived after a severe illness. The test was a complete failure.

CHART IV

<u>Nr.</u>	<u>Pre-Treatment</u>	<u>Infection</u>	<u>Results</u>
XV	7 July 3 ccm edema liquid from sheep XIV = sub-cut. in the left thigh	11 July 5424 Bacilli, then 2080 spores, subcut. on the right thigh	13 July slight edema. Death during the night 13-14 July after 62-70 hrs. Very slight, partially purulent edema. Spleen not enlarged. Bacilli - overall, quantitative.
XVI	7 July 9 ccm edema like XV		13 July slight edema. Death on 13 July after 55 hrs. Same condition as XV

CHART IV (cont'd)

<u>No.</u>	<u>Pre-treatment</u>	<u>Infection</u>	<u>Results</u>
XVII	7 July 15 ccm edema like XV	11 July 5/24 Bacilli, then 2080 spores sub-cutaneously - on the right thigh	Death after 14 hrs. Bacilli abundant generally excessive. Otherwise like XV
XVIII	7 July 9 ccm of a mixture 1 part edema and 4 parts blood - subcut. left shank (thigh)		14 July slight edema. 15 July edema begins to form margin upward. Sick. 16 July - continuously sick. Edema hard with margins. 19 July edema, absorbed with the exception of a slight infiltrate. Recovered.
XIX	7 July, 15 ccm mixture. (no part edema and 4 parts blood subcut. - left thigh (shank)		10 July moderate edema. Death during night 13-14 July, after 62-70 hrs condition similar to XV
XX	7 July 5 ccm of similar mixture used on XIX		13 July - slight edema. Death 14 July after 74 hrs. Edema somewhat more developed, otherwise similar to XV.
XXI	- (control animal)		Already on 12 July edema. 14 July extremely intensified, hanging from shank like a sack. Death on 14 July after 77 hrs.

If the causes were investigated, they could not be attributed to poor suitability of the sheep-edema employed in the pre-treatment, nor to the manner of the inoculation. If that were the case, regardless of subsequent tests, no animal would have stayed alive. It is assumed hence, that the only cause was the brevity of the time between the pre-treatment and the inoculation. This is not without importance because it appears appropriate to apply the Pasteur immunization inoculation with the use of weakened live bacilli in the edema treatment. The Pasteur treatment assures that immunity appears only 8-12 days after the disappearance of the local symptoms of the disease. The absorption of the edema formed in a natural way during the Pasteur treatment coincides (equals) the

inoculation of the artificially obtained liquid from another animal. A longer period of time is required also for this assumption. Therefore, one can assume ahead of time that the period of immunity of the two methods is identical.

Another circumstance is noted in observing (studying) Test IV, i.e., the noticeably fast course of the disease of sheep XVII. In other words the animal receiving the highest (largest) quantity of edema. Also the lining animals succumbed sooner than the control animal, in proportion to the dose of edema. It appears advisable to discuss the tests of a larger scale.

The surrounding circumstances were unfavorable as the animals were exhausted after the hot journey, hence arrived in a poor condition, but were subjected to tests without a period allowed for recuperation. Two animals (XXIII and XXVI) had a cough right from the start and XIII displayed a decreasing appetite. Nevertheless the pre-treatment was endured, without any exceptions. During these tests the sterilized blood of sheep suffering from anthrax was analyzed. Some of the analyzed blood was mixed with edema. The breed of the sheep was the same as the one used in Test IV (see Chart V).

Nr. XXIII of these 10 sheep was a diseased animal. The dissection of which revealed an extremely old contamination (disease). The death of this animal was not surprising, as old experiences taught that ill and weakened animals not only contracted contagious diseases more easily, but also could not be immunized in some instances, and in others with great difficulty. Under favorable circumstances this animal

would not have been used for testing purposes and no breeder would have retained it in his herd, nor would he have given it inoculations. However, the fact that a previously diseased animal was inoculated with edema or a blood edema mixture without showing obvious damage indicated that the procedure as such is harmless. Harmlessness, as such, does not restrict (limit) the same quality in connection with live bacilli. This ensues from the conception of lysin in accordance with Kruse's views and is proven by the death of sheep XXIX. This, however, will be discussed later.

Sheep XXVI proves that the disease of an immunized animal affects the success of the inoculation. This animal, like XXVIII, suffered of diseased respiratory tracts and was the only one of the animals treated with edema which showed an adhesion to anthrax, without a disturbance of the general condition.

It is appropriate to make some general remarks here. Animals like sheep XXIII and XXVI are the types which cause difficulty for immunization against anthrax as well as against any other infectious diseases. Unable to develop the needed body (organic reaction) for the production of immunity, they not only deteriorate (damage) statistics of immunity success (the effectiveness of the old Pasteur and the new Sobernheim methods is not doubted), but also provoke failures of those methods, which employ live (cultures) pathogenic agents. This applies to anthrax as well as swine erysipelas. Inoculation losses following the here employed edema treatment can be excluded with safety, but not losses through infection, as long as the (striving) (aspiring) strived for "Anti-lysin formation" is lacking. In the animals

employed in Test V, there was great damage or visible organic symptoms of the disease and under normal conditions such animals were not considered. In other cases, however, the illness hindering the immunization can be negligible and overlooked. On the other hand, the puzzling, but evident organic condition known as "individual disposition" exists.

CHART V

<u>Nr.</u>	<u>Pre-Treatment</u>		<u>Infection</u>	<u>Result</u>
XXII	25 July 2.5ccm edema from Sheep XXI	All inoculations were made on the inside of the left shanks - sub-cutaneously	4 Aug, 17300 bacilli, of which 1120 spores sub-cutaneously in the right shank.	No reaction.
XXIII	25 July 7.5 ccm of a mixture 1 part edema and 2 parts blood of Sheep XXI			The animal coughed right at the start, (waning) declining appetite. Could not recuperate. Death approx. 46 hours after inoculation. On the place of injection slight purulent edema. Spleen moderately enlarged. In the lower lobe of the left lung a not-sized abscess filled with pus, surrounded with hepatic lung tissue. Purulent slimy secretion from Nose nose. Excessive Anthrax bacilli in blood and in organs.
XXIV	25 July 2.5ccm Edema like XXII 30 July similar			No reaction
XXV	25 July 7.5ccm of a mixture like XXIII 30 July similar			Same as above
XXII	25 July 5ccm edema like XXII			The animal coughed right at start and was ill. Later, though the cough continued, obvious recuperation. On 7 Aug slight edema which hardened on 8 Aug and on 10 Aug disappeared, leaving just a slight infiltrate. The general condition did not show any evident disturbance.

CHART V (cont'd)

<u>Nr.</u>	<u>Pre-treatment</u>	<u>Infection</u>	<u>Result</u>
XXVII	25 July 15ccm mixture like XXIII	4 Aug. 17,300 bacilli, of which 1120 spores subcutaneously in the right shank.	No reaction
XXVIII	25 July 5ccm edema like XXII 30 July same		Same
XXIX	25 July 10ccm edema like XXII		Death 40-46 hrs after infection. On inoculation spot no evident reaction. Spleen enlarged, slight but distinct. There and in blood bacilli, somewhat less than customary to anthrax.
XXX	25 July 7.5ccm blood from sheep XXI 30 July same		On the 2nd and 3rd after the inoculations the animal was severely ill; slight edema appeared on 8 August, but disappeared on the following day. On 10 August the animal recuperated, just slightly diminished appetite. On 15 Aug. complete recovery.
XXXI (control animal)	-		On day after inoculation diffuses edema, which did not increase much. Death after 56 hours of typical anthrax.

One may progress one step further, and assume right at the start that such animals belong to a breed which are apt to contract pestilences of larger dimensions. They are vulnerable and without resistance to virus, existing only in small quantities. This (virus) increases in its quantity and pathogenicity and may be transmitted to other animals. These conditions are illustrated by the case of Sheep XXIII and must be taken into consideration.

Further, Sheep XXX deserves attention. This animal was pre-treated with sterilized anthrax blood exclusively and through this alone (became more resisting) increased its power of resistance. This evokes an historical, theoretic and practical interest (concern). First, because it confirms a very old observation by Toussaint. It is generally recognized that since Pasteur, the inoculation introduced by Toussaint, (employing warmed up anthrax blood) in cases when it is successful, merely implies that there is a weakening in the Bacilli which remained alive. In other words this method merely represents an incomplete Pasteur process. Here there is no question of live bacilli. The theoretic significance implies that in accordance with the Lysin Theort, Lysin must be present in the body, although in the first place of injection and particularly there were a special resistance of immunization exists, the quantity of lysin would be more strongly pronounced. Therefore, ~~through~~ though an immunizing effect of the blood was existant, it was so weak that the treated animal managed to survive only after a severe illness. One cannot attempt, hence, to employ (utilize) blood alone for the immunization. Nevertheless, it can be attempted in a conjunction (mixture) with edema and this is the ~~practically important~~ practically important side of the question, which provoked the mentioned tests with edema and blood mixtures. Though each animal can offer only a limited amount of edema, it is possible to extract (take) a larger quantity of blood from anthrax diseased animals. Hence, by mixing both substances a considerable quantity of serum may be obtained.

Besides, this consideration became invalid, relative to the economical manner of obtaining serum, since Test V proved that only a small quantity of serum was required for the immunizations. Factually, Nr. XXII was completely immunized by a single 2½ ccm inoculation. The repeated injections of the same quantity used on Nr. XXIV could not achieve ^{better} both results.

Nevertheless for further tests - be it that only a single edema treatment provides a long lasting immunity - one must reckon with a series of injections. This is closely connected with the characteristics of the edema, as Tests IV and V clearly indicate. During both tests the animals which received the largest quantity of edema died remarkably sooner than the controlled animals. Regarding Test IV, the foregoing remarks indicated that the infection (inoculation) of the treatment was premature. The premature death of the animal which received 15 ccm edema can be simply explained: at the time when the injection of bacilli was effected there was still free lysin (uncombined or loose) circulating in the blood and the (settlement) colonization of the anthrax was favorable due to the nullification of the normal protecting contrivances. As far as the other phases of the test show, though not quite as distinctly, one must assume that here the slighter quantity of edema was insufficient to produce the lytic effect or that this time a digestion (consumption) of the injected edema had taken place, which did not form (develop) a sufficient quantity of Anti-lysin.

The latter hypothesis could carry greater probability: first, because Sheep XVIII of the IV th test remained alive and further

because test V indirectly refers to such conditions. Here the time lapse between the first treatment and the (infection) injection was sufficiently long, in order to enable a number of sheep to develop the necessary immunity (protection). Only the animal which received the largest quantity of edema could not survive. This proves that the body of a normal animal is able to consume only a certain quantity of edema in a specific time; should this be exceeded (surpassed), instead of developing an immunity (through anti-lysin) an over sensitivity is produced (through existence of the injected lysin).

As Sheep XXVIII clearly indicates, it is not a question of the quantity of edema, but the manner in which it is administered. This animal has also received 10 ccm of the same edema, but in two separate injections. This offers a completely new revelation (manifestation) for the study of immunity which can also be proved in the rabbit test, though not half as clearly. The edema of animals suffering from anthrax, (when the largest part of the bacilli is destroyed and eliminated) represents a liquid which in itself is completely harmless for normal animals and which does not possess the characteristics (ingredients) of a poison. After injection of this liquid in specific quantities, (not too large) immunity against severe bacilli infection is reached within 8-10 days. However, if the time lapse is too brief, or if the specific quantity of edema is augmented, though the time is correct, the body is unable to consume (digest) it and an over-sensitivity develops. On numerous occasions one has attempted to detect the poisons of a pathogenic agent in the body juices of an

infected animal, without reaching favorable results. The diseased body ^ujuices are actually non-poisonous in themselves, not only with anthrax. Their characteristics, however, appear when they merge with the bacilli. As this could be established for pathogenic agents, which appear to have varied effects within the animal, it must be a question of a general rule (an overall law).

On the whole the clearest manifestation is the favorable lytic effect prompted by the infection. (On the whole the clearest manifestation is the infection influenced by the favorable lytic effect.) So far this has been proved with anthrax, tuberculosis (Viennese Clinical Weekly Research 1904 Nr. 30) and typhus. With the latter the lysin effect is clearly seen in various ways: 1) Non-fatal (even when not particularly small) doses of bacilli become fatal when merges with the otherwise harmless Lysin. 2) In employing fatal quantities, the dissection report changes due to the influence of Lysin, viz. the apportionment of cells of an "intraperitoneally" infected guinea pig. 3) The Lysin may destroy the immunity, when merges with bacilli, which was deluted with a "bactericidal immune serum". (2) The Lysin can, in merging with bacilli, lose the acquired immunity through an injection of a "bactericidal immune serum"). The more detailed information of these tests pertinent to typhus, which could not remain without influence on the absorption of the "organisms"(?) of the typhus immunity, may (follow around Christmas) be submitted by Christmas. The same quantity loads, without fail, to the desired immunity, as long as it is introduced separately, that is when a certain immunization exists against "harmless anthrax poison" contained in the edema.

(Further details of the report about the advantages of the method and the stability (durability) of the edema, etc. have so far no immediate interest, and should be reported as a summary of all tests.)